Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
4(3):507-513
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

Study of CD4, CD25 expression in transfusion dependent beta thalassemia patients

Aida S. Omar[1], Hoda A. Hassab[2], Abeer S. Elhadidi[1], Eman K. sobh[1]

[1]*Department of Clinical and chemical pathology Faculty of Medicine, University of Alexandria,*
[2]*Department of pediatrics Faculty of Medicine, University of Alexandria*

Email: emankhalifa17@gmail.com

ABSTRACT

Repeated blood transfusions in patients with β-thalassemia cause multiple antigenic stimuli that might change the Treg cells percentage. Tregs have become popular subjects of immunological research in recent years, because of their critical role in maintenance of immunological self tolerance and negative control of pathological and physiological immune response. The aim of work was to study the expression of CD4, CD25 in transfusion dependent beta thalassemia patient. This study was carried out on 80 subjects divided into three groups: GroupI; included 35 thalassemia major patients, group II; included 25 thalassemia intermediate patients attending at pediatric hematology clinic in Alexandria University Hospital and group III; included 20 healthy control children from general population. Statistically significant differences were found between group I and group III as regards CD4+CD25+ expression (p= 0.046) and CD4+CD25^{bright} expression (p=0.046). CD4+CD25+ expression has inverse relationship with the frequency and magnitude of non-hemolytic transfusional febrile reaction. CD4+CD25+and CD4+CD25^{bright} expression is higher in thalassemia major than healthy control. CD4+CD25+and CD4+CD25^{bright} have a role in immune tolerance to blood transfusion.

Keywords: Beta thalassemia, Treg cells, non-hemolytic febrile reaction.

INTRODUCTION

Beta Thalassemia major is an autosomal recessive inherited disorder resulting from mutations of genes involved in the synthesis of β globin chain. The children with this disorder become symptomatic due to progressive hemolytic anemia during the first year of life and must receive repeated blood transfusions to prevent life-threatening anemia[1]. A wide spectrum of immune abnormalities including both quantitative and functional defects has been reported by numerous studies in thalassemia patients with multiple transfusions. The abnormalities observed are changes in T- and B-lymphocyte subsets and functions[2-5].These defects have been attributed to the thalassemia itself and/or the applied therapeutic interventions[6].

Naturally occurring CD4+CD25+ regulatory T cells (Treg cells), which have become a popular subject of immunological research in recent years, play a crucial role in the maintenance of immunological self-tolerance and negative control of various immune responses to non-self antigens[7].Repeated blood transfusions of patients with β-thalassemia major cause multiple antigenic stimuli that might change the Treg cells expression. Induction of immune tolerance to prevent allo-immunization during transfusion is a potential approach to be explored. The identification of T regs to induce immune tolerance has opened possibility of developing novel immunotherapies in suppression of pathogenic immune response in immune diseases, transplantation and blood transfusion.[8]

Patients and methods:

Patients were attending at pediatric hematology clinic of Alexandria University Hospital from november2014 to April 2015. Healthy control from general population.

Inclusion and exclusion criteria:

How to Site This Article:
Aida S. Omar, Hoda A. Hassab, Abeer S. Elhadidi, Eman K. Abdallah. (2016). Study of CD4, CD25 expression in transfusion dependent beta thalassemia patients. *Biolife*. 4(3), pp 507-513.

DOI: 10.5281/zenodo.7332898
Received: 5 July 2016;
Accepted; 23 August 2016;
Available online : 4 September 2016

The study included patients diagnosed as β thalassemia (confirmed by hemoglobin electrophoresis), their ages were up to 14 years and they were on regular transfusion programs. The study excluded patients has any immunological disease transfusion and non-transfusion dependent β thalassemia.

Methods

All selected cases were subjected to full history taking (age, sex, age of first transfusion, interval between transfusions and febrile reaction to transfusion), complete clinical examination and measurement of CD4, CD25 expression.

Laboratory analysis:

Three ml of venous blood were collected from every individual participating in the study and were transferred in a tube containing K_2EDTA to perform flowcytometric assessment of CD4+ and CD25+ and CD4+ and $CD25^{bright}$ cells & for complete blood count (CBC) and blood film for differential WBCs count. Flowcytometry was based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes. The direct immunofluorescence was employed using labeled Abs. Immunofluorescence on the viable cells in suspension was analyzed using Becton Dickinson, FACS caliber flow cytometer equipped with Cell Quest Pro software. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5 % level.

RESULTS

In the present study cases were divided into 3 groups: group I thalassemia major patients (n=35), group II thalassemia intermedia (n=25) and group III healthy control (n=20).

The age of the group I ranged from 2–14 years with mean of 7.23 ± 3.44 years, the age of the group II ranged from 4 – 14years with mean of 8.48 ± 3.25 years while in the group III the age ranged from 2– 12 years with mean of 7.30 ± 3.37years. There was no significant difference between the mean age of the patients and that of the controls. As regards sex, males accounted for 54%of group I 46% of group II and 50% of group III and the rest were females.

There was no significant difference between the three groups as regard sex. (Table1, figure1,2)

Figure-1. Comparison between three groups according to Age (years)

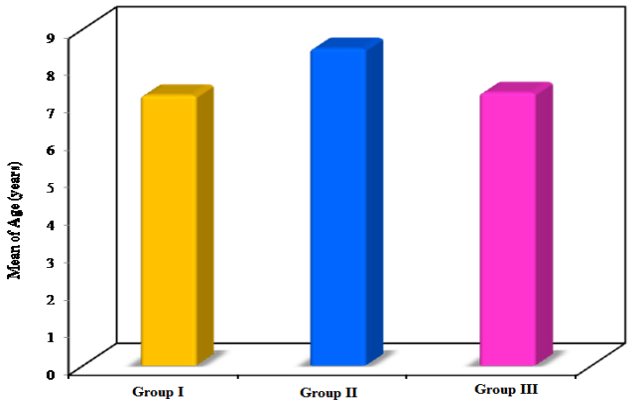
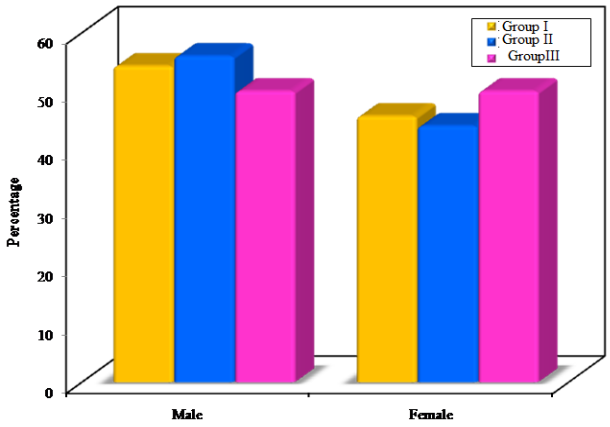


Figure-2. Comparison between three groups according to Sex



The difference between CD4+ CD25+ expression in group I and group II was not statistically significant as the means were11.57 ± 7.28% and 9.56 ± 5.45% respectively.(p_1=0.303). Also, the difference between was group II and group III was not statistically significant as the means of group III was 5.90± 2.56% (p_3=0.088).But, the difference between group I and

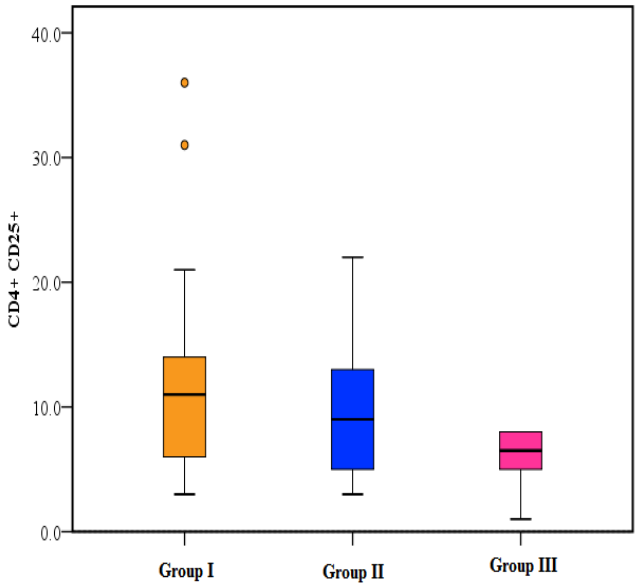
Table-1. Comparison between three groups according to sex and age

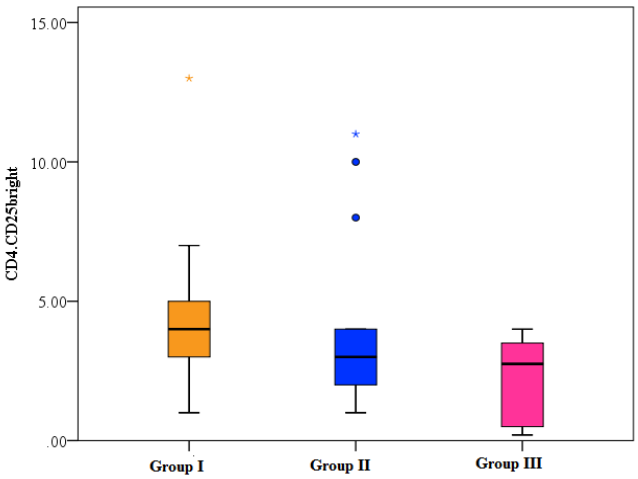
	Group-I (n = 35)		Group-II (n = 25)		Group-III (n = 20)		Test of sig.	p
	No.	%	No.	%	No.	%		
Sex								
Male	19	54	14	56.0	5	50.0	χ^2=104	0.950
Female	16	46	11	44.0	5	50.0		
Age (years)								
Min. – Max.	2.0 – 14.0		4.0 – 14.0		2.0 – 12.0		F= 1.0855	0.344
Mean ± SD.	7.23 ± 3.44		8.48 ± 3.25		7.30 ± 3.37			

χ^2: Chi square test
F: F test (ANOVA)
*: Statistically significant at p ≤ 0.05

group III was statistically significant (p_2=0.015).Also, there was overall statistical significant difference between the three groups. (p= 0.046). Regarding CD4+CD25^{bright}expression, difference between group I and group II was not statistical significant as the means were3.94 ± 2.22% and 3.58 ± 2.55% respectively.(p1=0.205).Also, between group II and group III as the mean of group III was 2.19 ± 1.47% (p3=0.141).But, there was statistical significant difference between group I and group III. (p2=0.017).Also, there was overall statistical significant difference between the three groups. (p= 0.046) **(**Table 2, figures3-7**).**

Figure-3. Comparison between three groups according to CD4 + CD25 $^{+}$ expression





Regarding non hemolytic transfusional febrile Reaction, there was statistical significant relation between CD4+CD25+ expression and febrile reaction in group I, the mean was lowest among those who had always febrile reaction to blood transfusion (6.50 ± 2.66%), followed by those who sometimes developed febrile reaction (8.13 ± 3.83%), followed by who rarely developed febrile reaction (11.86 ± 2.91%) and the highest was in those who never developed febrile reaction to transfusion (15.57 ± 9.36%). (P was 0.019)

Also in group II, there was significant relation between CD4+CD25+ expression and febrile reaction in, the mean was lowest among those who had always febrile reaction to blood transfusion (3.75 ± 0.96%),followed by those who sometimes developed febrile reaction (6.20 ± 2.17%), followed by who rarely developed febrile reaction (9.86±4.95%), and the highest was in those who never developed febrile reaction to transfusion (13.78±4.89%)(P was 0.004)

Table-.2. Comparison between three groups according to CD4+ CD25+ expression & CD4 + CD25 ^{bright} expression.

	Group I (n = 35)	Group II (n = 25)	Group III (n = 20)	KW_{X^2}	p
CD4+ CD25+					
Min. – Max.	3.0 – 36.0	3.0 – 22.0	1.0 – 8.0	6.175*	0.046*
Mean ± SD.	11.57 ± 7.28	9.56 ± 5.45	5.90 ± 2.56		
Sig. bet. Grps.	p_1=0.303, p_2=0.015*, p_3=0.088				
CD4 + CD25 bright					
Min. – Max.	1.0 – 13.0	1.0 – 11.0	0.20 – 4.0	6.179*	0.046*
Mean ± SD.	3.94 ± 2.22	3.58 ± 2.55	2.19 ± 1.47		
Sig. bet. Grps.	p_1=0.205, p_2=0.017*, p_3=0.141				

KW:Kruskal Wallis test sig.bet.Grps.Using Mann Whitney test
p_1 : p value for comparing between group |Iand groupII
p_2 : p value for comparing between group I and groupIII
p_3 : p value for comparing between groupII and groupIII
*: Statistically significant at p ≤ 0.05

Figure-4. Comparison between three groups according to CD4 + CD25 ^{bright} expression

Figure-5. Relation between CD4+CD25+ expression and reaction in group I and group II

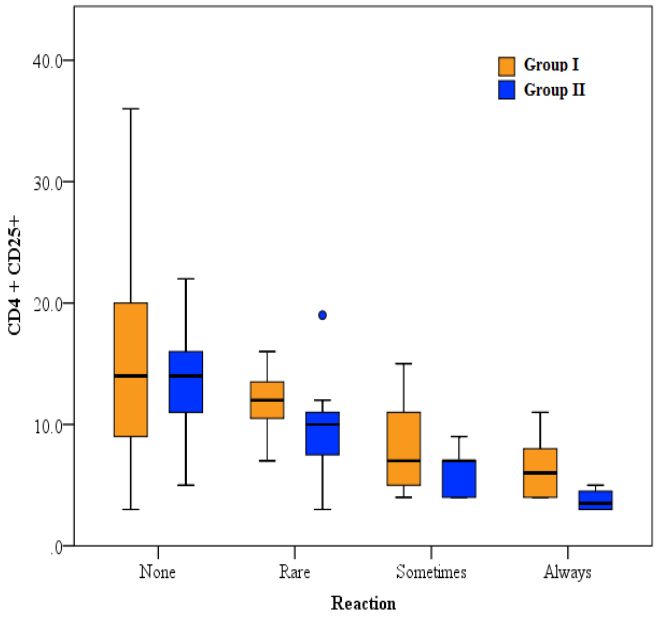


Figure-6. CD4+CD25$_+$expression and CD4+CD25^{bright} in a case of thalassemia major (group I)

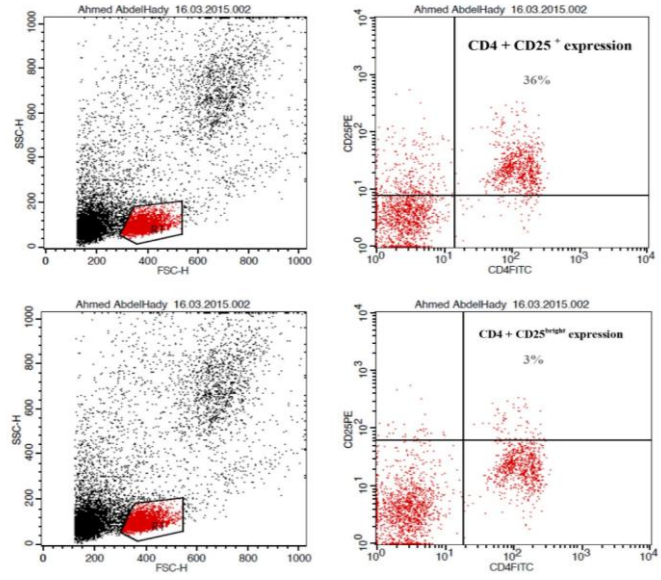


Figure-7: CD4+CD25$_+$expression and CD4+CD25^{bright} in a case of thalassemia intermediate (group II)

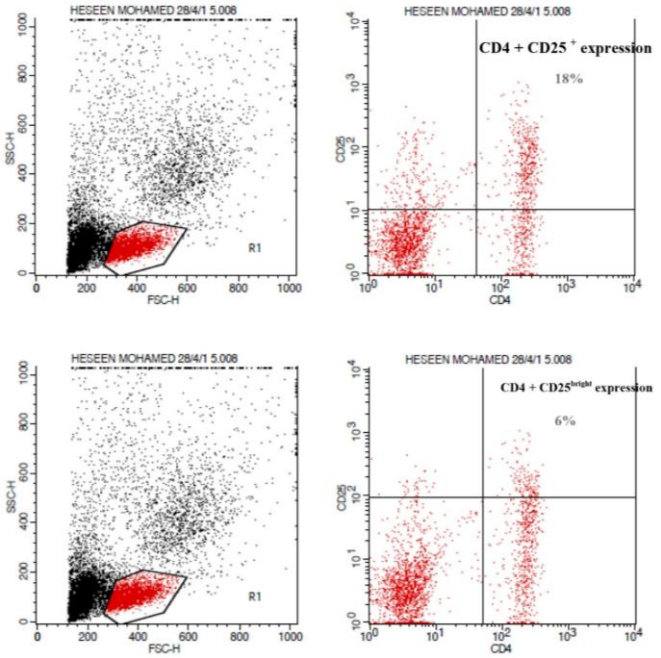


Figure-7. CD4+CD25$_+$ expression & CD4+CD25^{bright} in a healthy control (group III)

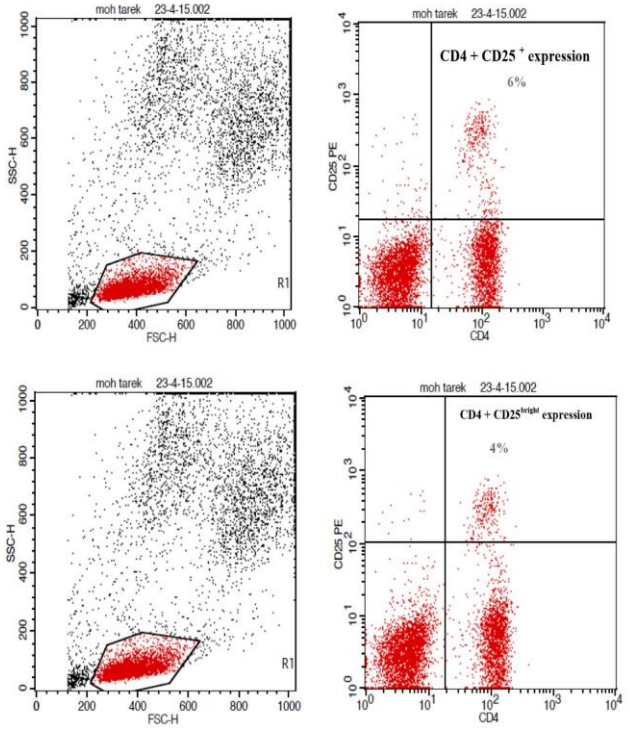


Table-3. Relation between CD4 + CD25+expression and febrile reaction in group I and group II

		N	CD4 + CD25+		Test of sig.	p
			Min. – Max.	Mean ± SD.		
Group I	**Reaction**				$^{KW}\chi^2$= 9.904*	0.019*
	None	**14**	3.0 – 36.0	15.57 ± 9.36		
	Rare	**7**	7.0 – 16.0	11.86 ± 2.91		
	Sometimes	**8**	4.0 – 15.0	8.13 ± 3.83		
	Always	**6**	4.0 – 11.0	6.50 ± 2.66		
Group II	**Reaction**				$^{KW}\chi^2$=13.411*	0.004*
	None	**9**	5.0 – 22.0	13.78 ± 4.89		
	Rare	**7**	3.0 – 19.0	9.86 ± 4.95		
	Sometimes	**5**	4.0 – 9.0	6.20 ± 2.17		
	Always	**4**	3.0 – 5.0	3.75 ± 0.96		

KW: Kruskal Wallis test, Z: Z for Mann Whitney test, *: Statistically significant at p ≤ 0.05

DISCUSSION

The results of the present study showed that, there was no significant difference between the three groups as regard age and sex. The difference between CD4+ CD25+ expression in group I and group II was not statistically significant as the means were11.57 ± 7.28% and 9.56 ± 5.45% respectively.(p1=0.303). Also, the difference between was group II and group III was not statistically significant as the mean of group III was 5.90± 2.56% (p3=0.088).But, the difference between group I and group III was statistically significant (p2=0.015). Also, there was overall statistical significant difference between the three groups. (p= 0.046)

In accordance with us, a study by Gunseli et al 2009[9], which was conducted on 30 thalassemia major patients and 30 thalassemia trait persons and 20 healthy age matched healthy control, the age of their patients from 1.5 years up to 22 years of age. They observed that CD4+CD25+ expression in thalassemia major and thalassemia trait patients was not statistically significant. Statistically significant difference was observed only between thalassemia major patients and controls (P =.0001). They attributed this finding to multiple antigenic stimuli that might increase the of CD4+CD25+ expression.

This is in agreement with Weili et all 2014[10]conducted a study in which Tregs and T helper responses were analyzed in Thalassemia mouse model (n=5) and WT (wild type) (n=5) spleens. The effect of allogeneic transfusions on Treg and T helper responses was also measured. The proportions of CD4+CD25+ Tregs were significantly higher (p<0.0001) in thalassemia model compared to WT mice before transfusion. Also, higher frequency of CD4+CD25+ (p = 0.002) and Tregs (p = 0.002) in Thalassemia model compared to WT mice after transfusion. They suggested that blood transfusion may stimulate expansion of T effector cells and also a counter regulatory response Tregs are expanded to dampen any potential collateral damage by effector cells.

In contrast with our findings, weili et al 2011[11], determined the CD4+ CD25+ expression in chronically transfused patients (22 sickle cell disease adult patients and 58 β thalassemia major adult patients). They found no differences in Treg frequencies between the transfused patient groups and healthy controls. This finding may be attributed to their selection of older age groups (from 13-29 years of age) than that of our study as we started by the age of 2 years up to age of 14 years old.

In our study, CD4+CD25^{bright} expression, difference between group I and group II was not statistical significant as the means were3.94 ± 2.22% and 3.58 ± 2.55% respectively.(p_1=0.205). Also, between group II and group III as the mean of group III was 2.19 ± 1.47% (p_3=0.141).But, there was statistical significant difference between group I and group III. (p_2=0.017).Also, there was overall statistical significant difference between the three groups. (p= 0.046)

In accordance with this finding, Gunseli et al 2009[9] observed that CD4+ CD25^{bright} expression in thalassemia major and thalassemia trait patients was not statistically significant. But, a statistically significant difference was observed only between thalassemia major patients and controls. (P =.0001).

In the current study, there was statistical significant relation between CD4+CD25+ expression and febrile reaction in group I and group II, the mean was lowest among those who had always febrile reaction to blood transfusion (6.50 ± 2.66% in group I and3.75 ± 0.96% in group II), followed by those who sometimes developed febrile reaction (8.13 ± 3.83% in group I and 6.20 ± 2.17% in group II), followed by who rarely developed febrile reaction (11.86 ± 2.91% in group I and9.86 ± 4.95% in group II) and the highest was in those who never developed febrile reaction to transfusion (15.57 ± 9.36% in group I and13.78 ± 4.89% in group II). P was 0.019 in group I and P was 0.004 in group II. This finding prove that Tregs have a role in tolerance to blood transfusion.

In agreement with Jin et al at 2007 [13], who conducted his study on mice. By detection T reg expression in mice prior to transfusion of allogeneic RBCs from transgenic mice expressing human glycophorin A (GPA) blood group antigens and measured alloantibody production. They concluded that Tregs do indeed participate in regulation of RBC alloantibody responses as they have role in

prevention of RBC alloimmunization and induction of transfusion tolerance to blood transfusion. Also, they reported that depletion of CD25-expressing cells with anti-CD25 prior to transfusion further enhanced alloantibody production indicating that naive T regs participate in regulation of transfusion associated antibody responses to multiple allogeneic RBCs.

Also weili et al at 2009 [14] who conducted their study on mice, by weekly transfusion of allogeneic RBCs from transgenic mice expressing human glycophorin A (GPA) blood group antigens, more than 50% of mice developed alloantibodies after 4 weeks, Tregs from non-responders (without alloantibodies) and responders (with alloantibodies) suppressed proliferation of effector T cells, but T regs from non-responders were better suppressors as indicated by lower proliferation rates of effector T cells. So, rates and frequency of alloimmunization due to multiple transfusions is controlled by T regs.

In addition, weili et al at 2011[11], they determined the Treg expression chronically transfused patients (22 sickle cell disease adult patients and 58 β thalassemia major adult patients with and without alloantibodies). They found reduced Treg activity in alloantibody responders compared with non-responders cultures of CD4+CD25^{bright}Tregs and CD4+CD25– T effector cells. Proliferation rates of T effector cells were lower in non-alloimmunized SCD or TM patients compared with those of alloimmunized sickle patients ($P < 0.05$). These data indicate that CD4+CD25^{bright}Tregs from non-alloimmunized sickle cell disease or thalassemia major patients can suppress proliferation of T effector cells more effectively than Tregs from alloimmunized SCD patient. They have found reduced peripheral Treg function in transfused antibody responders compared with non-responders similar to what we previously reported in mice.

In contrast, Benoît et al 2015[15] compared the CD4+ T-cell phenotypes and functions between a group of sikle cell disease patients(n = 11) who never became immunized and a group of SCD patients (n = 10) who had become immunized, they did not find any difference between Treg-cell phenotypes or functions in alloimmunized and nonalloimmunized patients. This finding may be attributed to small sample size.

Also, Robert et al 2015[16] conducted their study on 90 children with SCD on chronic RBC transfusion therapy. They stated that there was no statistical significant difference in the percentages of Tregs in Alloimmunized (n=11) and non-alloimmunized Patients (n=55) on Chronic Transfusion as p= 0.43. This finding may be attributed to racial difference

CONCLUSION

In this study CD4+CD25+and CD4+CD25^{bright} expression is higher in thalassemia major than healthy control. This finding is attributed to multiple antigenic stimuli during blood transfusion. Tregs have a role in tolerance to blood transfusion like that in tolerance of organ transplantation.

Acknowledgements

Authors' contributions: Concept, design, definition of Intellectual content, data acquisition, data analysis, statistical analysis, and clinical studies: Eman K. Sobh, Abeer S. Elhadidi literature search, manuscript preparation, manuscript review, and manuscript editing: Abeer S. Elhadidi, experimental studies: Hoda M.A hassab. All authors have read and approved the final version of the manuscript

Conflicts of interest

Authors declare that there is no conflict of interests regarding the publication of this paper**.**

REFERENCES

[1]. DeBaun M, Vichinsky E. Hemoglobinopathies. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF (eds). Nelson Textbook of Pediatrics. 18th ed. Philadelphia, PA: Saunders Elsevier; 2007:2025–38.

[2]. Dwyer J, Wood C, McNamara J, Williams A, Andiman W, Rink L, et al. Abnormalities in the immune system of children with betathalassemia major. Clin Exp Immunol 1987;68(3):621-9.

[3]. Khalifa AS, Maged Z, Khalil R, Sabri F, Hassan O, el-Alfy M. T-cell function in infants and children with beta-thalassemia Acta Haematol 1988; 79:153–5.

[4]. Ezer U, G¨ulderen F, Culha VK, Akgül N, Gürbüz O. Immunological status of thalassemia syndrome. Pediatr HematolOncol 2002; 19:51–8.

[5]. Dua D, Choudhury M, Prakash K. Altered T and B lymphocytes in multitransfused patients of thalassemia major. Indian Pediatr 1993; 30:893-6.

[6]. Farmakis D, Giakomis A, Polymeropolus E, Aessopos A. Pathogenetic aspects of immune deficiency associated with beta-thalassemia. Med Sci Monit 2003; 9:19–22.

[7]. Corthay A. How do regulator T cells work? Scand J Immunol 2009; 70:326–36.

[8]. Sakaguchi S. Naturally arising CD4^{+} regulatory T cells for immunologic self-tolerance and negative control of immune responses. Ann Rev Immunol 2004; 22:531–62.

[9]. Gunseli B, Ela G, Yanikkaya D, Yildiz Y. The role of treg cells and foxp3 expression in immunity of β-thalassemia major and β-thalassemia trait. Pediatric Hematology and Oncology 2010; 27:534–45.

[10]. *Sateesh Pujari and* Estari Mamidala *(2015). Anti-diabetic activity of Physagulin-F isolated from* Physalis angulata *fruits.* The Ame J Sci & MedRes*,2015,1(1):53-60. doi:10.17812/ajsmr2015113.*

[11]. Weili B, Hui Z, Karina. Immunologic characterization suggests reduced alloimmunization in a murine model of thalassemia intermedia. Transfusion 2014; 54:2880-91.
[12]. Weili B, Hui Z, Xiaojuan L, Margaret T, Joseph S, Sujit S, et al. Immune regulation in chronically transfused allo-antibody responder and Non responder patients with sickle cell disease and b-thalassemia major. Am J Hematol 2010; 86: 1001–6.
[13]. Benoît V, Marie T, Maxime D, Sadaf P, Rahma E, Anoosha H, et al. Partial dysfunction of Treg activation in sickle cell disease. Am J Hematol 2014; 89:261–6.
[14]. Jin Y, Susanne H, Karina Y. Prevention of red cell alloimmunization by CD25 regulatory T cells in mouse models. Am J Hematol 2007; 82:691–6.
[15]. Weili B, Jin Y, Susanne H, Karina Y. Regulatory T-cell status in red cell alloimmunized responder and non-responder mice. Bloodjournal 2009; 22:5624-7.
[16]. Benoît V, Marie T, Anoosha H, Sadaf P, Julie R, Rahma E, et all. Phenotypic differences of CD4+ T cells in response to red blood cell immunization in transfused sickle cell disease patients. Eur J. Immunol 2015; 45: 1868–79.
[17]. Robert S, John T, Ross M, Erin M, Cassandra D, Anne M, et al. Immunophenotypic parameters and RBC alloimmunization in children with sickle cell disease on chronic transfusion. Am J Hematol 2015; 90:1135-41.